

Entry of the amendments are respectfully requested.

Claim Amendments

Claims 24, 29, 34, 35, 36 and 38 have been amended to more clearly define the invention. Specifically, the claims have been amended to recite that the nucleotide positions of SEQ ID NO: 1 are numbered from the putative initiation codon. Support for these amendments can be found throughout the Specification, for example, on page 7, lines 9-10. No new matter has been added.

Entry of the amendments are respectfully requested.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned at (978) 341-0036.

Respectfully submitted,  
HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

By 

Brian P. Turano  
Registration No. P-50,734  
Telephone: (978) 341-0036  
Facsimile: (978) 341-0136

Concord, MA 01742-9133

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## MARKED UP VERSION OF AMENDMENTS

Specification Amendments Under 37 C.F.R. § 1.121(b)(1)(iii)

Replace the paragraph at page 5, lines 3 through 15, with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

The invention further relates to a method of diagnosing or aiding in the diagnosis of neurodegenerative disease in an individual comprising obtaining a nucleic acid sample from the individual and determining the nucleotide present at nucleotide position 5254 of SEQ ID NO: 1, wherein the nucleotide position is numbered from the putative initiation codon, wherein presence of a thymine at said position is indicative of increased likelihood of neurodegenerative disease in the individual as compared with an appropriate control, *e.g.*, an individual having a cytosine at said position. The invention also relates to a method of diagnosing or aiding in the diagnosis of neurodegenerative disease in an individual comprising obtaining a nucleic acid sample from the individual and determining whether there is a deletion of a thymine at nucleotide position 6594 of SEQ ID NO: 1, wherein the nucleotide position is numbered from the putative initiation codon, wherein deletion of a thymine at said position is indicative of increased likelihood of neurodegenerative disease in the individual as compared with an appropriate control, *e.g.*, an individual who does not have a deletion at said position.

Replace the paragraph at page 5, lines 16 through 26, with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

The invention also relates to a method of treating a neurodegenerative disorder associated with the presence of a thymine at nucleotide position 5254 of SEQ ID NO: 1 in an individual, wherein the nucleotide position is numbered from the putative initiation codon, comprising administering to the individual an agent selected from the group consisting of a polypeptide encoded by SEQ ID NO: 2 or an active portion thereof, a nucleic acid molecule which encodes SEQ ID NO: 2

or an active portion of SEQ ID NO: 2, and an agonist of SEQ ID NO: 2. The invention further relates to a method of treating a neurodegenerative disorder associated with a deletion at nucleotide position 6594 of SEQ ID NO: 1 in an individual, wherein the nucleotide position is numbered from the putative initiation codon, comprising administering to the individual an agent selected from the group consisting of a polypeptide encoded by SEQ ID NO: 2 or an active portion thereof, a nucleic acid molecule which encodes SEQ ID NO: 2 or an active portion of SEQ ID NO: 2, and an agonist of SEQ ID NO: 2.

Replace the paragraph at page 5, line 27 through page 6, line 11, with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

The invention also encompasses a method of diagnosing or aiding in the diagnosis of neurodegenerative disease associated with the presence of a thymine at nucleotide position 5254 of SEQ ID NO: 1 in an individual, wherein the nucleotide position is numbered from the putative initiation codon, comprising obtaining a sample comprising a Spastin polypeptide from the individual and determining the size of the Spastin polypeptide, wherein if the Spastin polypeptide is significantly shorter than SEQ ID NO: 2 it is indicative of neurodegenerative disease. The invention also provides a method of diagnosing or aiding in the diagnosis of neurodegenerative disease associated with the presence of a deletion at nucleotide position 6594 of SEQ ID NO: 1 in an individual, wherein the nucleotide position is numbered from the putative initiation codon, comprising obtaining a sample comprising a Spastin polypeptide from the individual and determining the size of the Spastin polypeptide, wherein if the Spastin polypeptide is significantly shorter than SEQ ID NO: 2 it is indicative of neurodegenerative disease. In one embodiment, the Spastin polypeptide is significantly shorter than SEQ ID NO: 2 if the Spastin polypeptide comprises less than about 75% of the amino acids of SEQ ID NO: 2.

Replace the paragraph at page 9, line 26 through page 10, line 12, with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

To characterize the full sequence of the ORF and to identify potential disease-causing mutations, PCR products from ARSACS patient and control DNA were sequenced. The primers for these reactions are shown in Figure 7. A single-base deletion of a thymine at position 6594 (6594ΔT) (Figure 2A) was found on all copies of the major ancestral haplotype examined (a total of 32 chromosomes), but was absent in all chromosomes of carrier parents that were not transmitted to ARSACS offspring. This mutation causes a frame shift and results in a subsequent stop codon that truncates the final 43% of the predicted protein. A second mutation, a nonsense mutation of substitution of a thymine for a cytosine at nucleotide position 5254 (c5254T) (Figure 2B), wherein the nucleotide position is numbered from the putative initiation codon results in the substitution of a stop codon for an arginine and was found on the minor ARSACS haplotype carried in a heterozygous state (in *trans* to the major ARSACS mutation) in six patients from two families (5). Both mutations are thus completely associated with their respective core haplotypes and are predicted to have severe effects on the encoded protein. The presence of these two mutations provides strong evidence that mutations in this ORF are responsible for ARSACS. The gene is referred to herein as *spastin* (gene symbol: SPAS).

Replace the paragraph at page 10, lines 13 through 22, with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

In the course of the complete resequencing of the *spastin* gene in ARSACS patients, additional sequence variants were found which proved to be polymorphisms found on non-ARSACS-bearing chromosomes as well. These included four silent substitutions: substitution of a thymine for a cytosine at nucleotide position 3945, substitution of a cytosine for a thymine at nucleotide position 6603, substitution of a thymine for a cytosine at nucleotide position 7731, and substitution of a thymine for a cytosine at nucleotide position 10054 (C3945T, T6603C, C7731T and C10054T, respectively), and an amino acid-altering substitution of a cytosine for a thymine at nucleotide position 7856 (T7856C), wherein the nucleotide position is numbered from the putative initiation codon, that results in the substitution of an alanine for a valine in the predicted protein.

Replace the paragraph at page 12, line 24 through page 13, line 7, with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

Work described herein strongly supports that a frameshift and a nonsense mutation identified within the *spastin* gene cause ARSACS. Though the gene appears to be widely expressed, the truncation of the Spastin protein caused either by homozygous (6594ΔT/6594ΔT) or compound heterozygous (C5254T/6594ΔT) genotypes, wherein the nucleotide position is numbered from the putative initiation codon, apparently lead to symptoms predominantly affecting the nervous system. The high level of expression of *spastin* mRNA in the granular cell layer of the adult rat cerebellum is especially interesting in light of an earlier observation of the reduced thickness of the granular layer found during the postmortem examination of tissue from an ARSACS patient (Bouchard, J-P., *In: Handbook of Clinical Neurology 16: hereditary neuropathies and spinocerebellar degenerations*, pp.451-459, Elsevier Science Publishers, Amsterdam (1991)). Thus, the high mRNA expression levels seen in the CNS indicate a possibly unique role for Spastin in the genesis or maintenance of neural cell function.

Replace the paragraph at page 14, line 25 through page 15, line 15, with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

SEQ ID NOS: referred to herein are as follows. SEQ ID NO: 1 refers to the complete exon of the human *spastin* gene as shown in Figures 9A-9F. SEQ ID NO: 2 refers to the protein encoded by the ORF of SEQ ID NO: 1, particularly as shown in Figures 9A-9F and 5A-5C. SEQ ID NO: 3 refers to the complete exon of the murine *spastin* gene as shown in Figures 8A-8G. SEQ ID NO: 4 refers to the protein encoded by the ORF of SEQ ID NO: 3, particularly as shown in Figures 9A-9F and 5A-5C. SEQ ID NOS: 5 and 6 are intentionally omitted. SEQ ID NO: 7 refers to a nucleotide sequence which is identical to SEQ ID NO: 1 except for a deletion of a thymine at position 6594, wherein the nucleotide position is numbered from the putative initiation codon. SEQ ID NO: 8 refers to the protein encoded by the ORF of SEQ ID NO: 7. SEQ ID NO: 9 refers to a nucleotide sequence which is identical to SEQ ID NO: 1 except for a substitution of a thymine for a cytosine at position 5254, wherein the nucleotide position is numbered from the putative initiation codon. SEQ

ID NO: 10 refers to the protein encoded by the ORF of SEQ ID NO: 9. SEQ ID NO: 11, 12, 13 and 14 refer to nucleotide sequences which are identical to SEQ ID NO: 1 except for a substitution of a thymine for a cytosine at position 3945, substitution of a cytosine for a thymine at position 6603, substitution of a thymine for a cytosine at position 7731, and substitution of a thymine for a cytosine at position 10054, wherein the nucleotide position is numbered from the putative initiation codon, respectively. SEQ ID NO: 15 refers to a nucleotide sequence which is identical to SEQ ID NO: 1 except for substitution of a cytosine for a thymine at position 7856, wherein the nucleotide position is numbered from the putative initiation codon. SEQ ID NO: 16 refers to the protein encoded by the ORF of SEQ ID NO: 15. The sequences corresponding to all other SEQ ID NOS: used herein are shown throughout the application.

Claim Amendments Under 37 C.F.R. § 1.121(c)(1)(ii)

24. (Amended) A method of diagnosing or aiding in the diagnosis of neurodegenerative disease in an individual comprising:
- obtaining a nucleic acid sample from the individual; and
  - determining the nucleotide present at nucleotide position 5254 of SEQ ID NO: 1, wherein the nucleotide position is numbered from the putative initiation codon, wherein presence of a thymine at said position is indicative of increased likelihood of neurodegenerative disease in the individual as compared with an individual having a cytosine at said position.
29. (Amended) A method of diagnosing or aiding in the diagnosis of neurodegenerative disease in an individual comprising:
- obtaining a nucleic acid sample from the individual; and
  - determining whether there is a deletion of a thymine at nucleotide position 6594 of SEQ ID NO: 1, wherein the nucleotide position is numbered from the putative initiation codon.

wherein deletion of a thymine at said position is indicative of increased likelihood of neurodegenerative disease in the individual as compared with an individual who does not have a deletion at said position.

34. (Amended) A method of treating a neurodegenerative disorder associated with the presence of a thymine at nucleotide position 5254 of SEQ ID NO: 1 in an individual, wherein the nucleotide position is numbered from the putative initiation codon, comprising administering to the individual an agent selected from the group consisting of:
- a) a polypeptide encoded by SEQ ID NO: 2 or an active portion thereof;
  - b) a nucleic acid molecule which encodes SEQ ID NO: 2 or an active portion of SEQ ID NO: 2; and
  - c) an agonist of SEQ ID NO: 2.
35. (Amended) A method of treating a neurodegenerative disorder associated with a deletion at nucleotide position 6594 of SEQ ID NO: 1 in an individual, wherein the nucleotide position is numbered from the putative initiation codon, comprising administering to the individual an agent selected from the group consisting of:
- a) a polypeptide encoded by SEQ ID NO: 2 or an active portion thereof;
  - b) a nucleic acid molecule which encodes SEQ ID NO: 2 or an active portion of SEQ ID NO: 2; and
  - c) an agonist of SEQ ID NO: 2.
36. (Amended) A method of diagnosing or aiding in the diagnosis of neurodegenerative disease associated with the presence of a thymine at nucleotide position 5254 of SEQ ID NO: 1 in an individual, wherein the nucleotide position is numbered from the putative initiation codon, comprising:
- a) obtaining a sample comprising a Spastin polypeptide from the individual;
  - b) determining the size of the Spastin polypeptide,
- wherein if the Spastin polypeptide is significantly shorter than SEQ ID NO: 2 it is indicative of neurodegenerative disease.

38. (Amended) A method of diagnosing or aiding in the diagnosis of neurodegenerative disease associated with the presence of a deletion at nucleotide position 6594 of SEQ ID NO: 1 in an individual, wherein the nucleotide position is numbered from the putative initiation codon, comprising:
- a) obtaining a sample comprising a Spastin polypeptide from the individual;
  - b) determining the size of the Spastin polypeptide,
- wherein if the Spastin polypeptide is significantly shorter than SEQ ID NO: 2 it is indicative of neurodegenerative disease.